

STUDIES ON CARNATION YELLOWS, A VIRUS COMPLEX
CONSISTING OF CARNATION MOSAIC VIRUS AND
CARNATION STREAK VIRUS

BY

RALPH WOLFLEY AMES

B.S., University of Wyoming, 1939

M.S., University of Wyoming, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
PLANT PATHOLOGY IN THE GRADUATE COLLEGE
OF THE UNIVERSITY OF ILLINOIS, 1950

URBANA, ILLINOIS
1950



STUDIES ON CARNATION YELLOWS, A VIRUS COMPLEX
CONSISTING OF CARNATION MOSAIC VIRUS AND
CARNATION STREAK VIRUS

BY

RALPH WOLFLEY AMES

B.S., University of Wyoming, 1939

M.S., University of Wyoming, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
PLANT PATHOLOGY IN THE GRADUATE COLLEGE
OF THE UNIVERSITY OF ILLINOIS, 1950

URBANA, ILLINOIS

1950

STUDIES ON CARNATION YELLOWS, A VIRUS COMPLEX CONSISTING OF CARNATION MOSAIC VIRUS AND CARNATION STREAK VIRUS

Studies on carnation yellows (virus), carnation mosaic, and carnation streak were initiated in an effort to obtain information relative to three important phases of carnation virus diseases: (a) diagnostic tests for the detection of the disease in plants which may be symptomless or have questionable symptoms, (b) practical methods which can be used for the control of the diseases or the cure of affected plants, and (c) information of a fundamental nature regarding the properties and characteristics of the causal agents of the diseases.

These virus diseases of carnations cannot be detected by the use of Lindner's rapid chemical test, by Cochrane's chromatographic method, by Thomas and Mussenbrock's ultraviolet fluorescent test, or by the ultrachromatography technique of Thornberry.

Aqueous solutions of zinc sulfate (1:4000), calcium chloride (1:500, 2:500, 6:1000), ammonium molybdate (1:2000, 1:3000), trisodium phosphate (1:1000, 1:2000, 1:4000, 4:1000), and malachite green (1:4000) when applied to cut basal ends of carnation cuttings for forty-eight or seventy-two hours failed to control or prevent the diseases. Efforts to inactivate carnation mosaic virus *in vivo* by means of hot water therapy failed because the heat tolerance of the carnation cuttings is not as great as the heat tolerance of the carnation mosaic virus (60° C.).

The thermal inactivation time of carnation mosaic virus in juice expressed from diseased plants is between 63° C. and 68° C. for fifteen minutes. The most effective H-ion concentration for inoculating test plants with carnation mosaic virus by means of the carborundum technique is around pH 7.0. Infection by carnation mosaic virus does not occur at a pH greater than 9.0 or less than 4.0. A low concentration of carnation mosaic virus in the plant is indicated by the low dilution end point (1:1000) of the virus in sap expressed from diseased plants.

Carnation mosaic virus can be sufficiently purified and concentrated from the expressed juice of diseased carnation plants to detect the virus particles by electron microscopy. The virus particles

were first adsorbed and lodged on Celite by filtering the juice at pH 4.0. The particles were then removed from the Celite by thorough elution and solution with 0.1 molar phosphate buffer at pH 7.2. The resultant eluate was then subjected to differential centrifugation. It was first centrifuged at 50,000 x gravity for thirty minutes at approximately 0° C. The supernatant was discarded and the sediment resuspended in 12 ml. of 0.1 molar phosphate buffer and allowed to stand overnight at approximately 3° C. The liquid after being carefully decanted from the solids that sedimented by gravity overnight was centrifuged at 580 x gravity for fifteen minutes at approximately 0° C. The supernatant from this treatment was then centrifuged at 15,275 x gravity for fifteen minutes at approximately 0° C. The sediments obtained from this centrifugation were examined in the electron microscope. A sample of juice expressed from healthy carnation plants was treated in a manner similar to and simultaneously with the diseased tissue.

The sediments of the diseased and healthy carnations were prepared for electron microscopy in the usual manner and shadowed with palladium at an angle of 4 to 1 (25 degree angle). Preliminary observations and electromicrographs were made on a RCA-EMC-2 electron microscope at thirty kilovolts at a working magnification of 5,000 diameters. Electronmicrographs were also made at magnifications approaching 36,000 diameters on the RCA-EMB electron microscope at fifty kilovolts. Spherical particles were found in the material originating from the diseased carnations, whereas they were not detected in the material originating from healthy plants. The particles found in the diseased specimen were fairly numerous and well distributed over the microscopic field. The material containing the particles proved to be infectious when inoculated to leaves of *Dianthus barbatus*, whereas that originating from healthy plants and without demonstrable particles was not infectious.

The size of these spherical particles was determined by measuring 72 particles at a projected magnification of 400,000 and then calculating the arithmetic mean diameter. The particles are approximately 31 millimicrons in diameter. From these data it is concluded that these spherical particles constitute the active carnation mosaic virus responsible for the carnation mosaic disease.

VITA

Ralph W. Ames was born June 27, 1918 in Etna, Wyoming where he attended grade school. He attended high school in Afton, Wyoming. He was graduated from the University of Wyoming in 1939 with a degree of Bachelor of Science with honors in Liberal Arts and Sciences, and a Master of Science in Botany from the same institution in 1940.

He was a student teaching assistant at the University of Wyoming and a graduate teaching assistant and research assistant at the University of Illinois. He was also a fellow in horticulture for one year at the University of Illinois.

His other experiences include four years military service with the army medical department and about two years service with the Soil Conservation Service, U.S.D.A., first as a range examiner and later as a farm planner and work unit leader.

The author is a member of the Americal Phytopathological Society, Botanical Society of America, Mycological Society of America, The American Association for the Advancement of Science, The Soil Conservation Society of America, The Illinois Academy of Science, Phi Kappa Phi, Sigma Xi (associate), Phi Sigma, Phi Eta, Gamma Sigma Delta, Pi Alpha Xi, Gamma Alpha, Associated Cosmopolitan Clubs, and Lambda Delta Sigma.

Publications are:

Solheim, W. G. and R. W. Ames, The Effects of Some Natural Gasses upon Plants, J. Colorado-Wyoming Acad. Sci. 3: 38, 1941.

Ames, Ralph W., Therapy of Carnation Virus Diseases, Process of Publication in Proc. Ill. Acad. of Sciences.

